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Cross-Species Amplification of DNA Microsatellite Loci in an Australian Lineage of Social Lizards (Scincidae, Genus *Egernia*)

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Advances in molecular technology have led to the discovery of a number of lizard species living in stable family groups. These social lizards provide an opportunity to test current hypotheses about the evolution and maintenance of vertebrate social systems developed using endothermic models (i.e., birds and mammals). As such, social lizards have increasingly been the focus of intense research, in particular studies within the Australian *Egernia* Group lineage of skinks.

The scincid genus *Egernia* comprises 32 species of medium to large-sized viviparous skinks and is endemic to Australia (Chapple 2003). Recently, much interest has been generated in this group due to the realization that complex social systems and monogamy are widespread within the genus, with reports on 26 of the 32 species suggesting social structures of varying complexity, ranging from predominately solitary through to large social aggregations in excess of 30 individuals (reviewed in Chapple 2003). Long-term behavioral and genetic studies on four species have shown that these large aggregations are stable between years and consist of closely related individuals (Chapple 2003). In addition, *E. cunninghami*, *E. saxatilis* and *E. stokesii* have been shown to exhibit long-term social and genetic monogamy, with *E. cunninghami* and *E. stokesii* exhibiting strong inbreeding avoidance (Gardner et al. 2001, 2002; O'Connor and Shine 2003; Stow et al. 2001; Stow and Sunnucks 2004a,b). Such behavioral traits are extremely rare in lizards (Bull 2000), therefore the genus provides a unique opportunity to examine the evolution of complex sociality within squamate reptiles and test existing hypotheses about the evolution of vertebrate sociality (Chapple 2003).

Several factors have enabled *Egernia* to be utilized as a 'model' system for examining the evolution of sociality and monogamy in lizards. First, most species are large, long-lived and exhibit a strong attachment to a home site (e.g., rock crevice, burrow, log) and therefore are well-suited to long-term behavioral and genetic studies (Chapple 2003). Second, a large number of microsatellite primers have been developed for *E. stokesii* (Gardner et al. 1999; 11 EST primers), *E. cunninghami* (Stow 2002; 5 Ecu primers) and the closely related *Tiliqua rugosa* (Cooper et al. 1997; 6 Tr primers). However, what has facilitated the research to date is a high degree of successful cross-species PCR amplification of microsatellite loci within the lineage using the same primer pairs. These primers have now been utilized for examining aspects of sociality and mating systems in *E. stokesii* (Gardner et al. 2001, 2002), *E. saxatilis* (O'Connor and Shine 2003), *E. cunninghami* (Stow et al. 2001; Stow and Sunnucks 2004a,b), *E. striolata* (Bonnett 1999; Bull et al. 2001), *E. whitii* (Chapple and Keogh 2006) and *E. frerei* (Fuller et al. 2005).

Because this is a burgeoning and active area of research, similar studies are expected to be conducted for most other *Egernia* species (Chapple 2003). However, at present there is a lack of detailed information on the cross-species amplification of available *Egernia* microsatellite primers (especially for those that were problematic for some species). Microsatellite markers are costly and time intensive to develop and successful cross-species amplification can represent a substantial reduction in cost and time. Detailed information on the potential utility of each microsatellite locus would aid in selecting primers for particular *Egernia* species, saving time and money. Here we report the cross-species application of the available microsatellite primers in five *Egernia* species. These species represent four of the six species groupings within the genus: *E. cunninghami* and *E. stokesii* (*cunninghami* group, 4 species), *E. saxatilis* (*striolata* group, 9 species), *E. whitii* (*whitii* group, 12 species) and *E. frerei* (*major* group, 4 species). Since the remaining two species groups comprise a total of three species (*E. kingii* group, 1 species; *E. luctuosa* group, 2 species), the four species groups that we examine contain 29 of the 32 species in the genus and therefore should provide valuable information for future researchers. In order to supplement our detailed analysis of five species we provide a brief summary of the results from published studies that have used microsatellites in *Egernia* species.

The PCR conditions used for each species are contained within the original published studies and therefore we only provide relevant information that has not been published elsewhere. For *E. stokesii* details regarding PCR conditions and parameters are contained in Cooper et al. (1997) and Gardner et al. (1999, 2000, 2001, 2002), with the conditions for the Ecu primers as detailed in Stow (2002). For *E. cunninghami* this information is provided in Stow et al. (2001), Stow (2002), and Stow and Sunnucks (2004a, b),

TABLE 1. Cross-amplification of the available microsatellite primers in the *Egernia*, *Tiliqua* and *Cyclodomorphus* species tested to date. P = polymorphic; M = monomorphic; U = unsuccessful amplification; ? = some degree of amplification (see Table 2 for further detail). Species codes as follows: EC = *E. cunninghami*, EF = *E. frerei*, EI = *E. inornata*, ESa = *E. saxatilis*, ESto = *E. stokesii*, EStr = *E. striolata*, EW = *E. whitii*, TA = *T. adelaidensis*, TR = *T. rugosa*, TS = *T. scincoides*, CB = *C. branchialis*, CC = *C. casuarinae*, CG = *C. gerrardii*.

Locus	EC	EF	EI	ESa	ESto	EStr	EW	TA	TR	TS	CB	CC	CG
Tr3.2	P	U[1]			P	P[4]	U	P[1]	P[6]	P[1]		P[1]	U[1]
Tr4.6	?	M?[1]			P[1]			M?[1]	P[6]	M?[1]		P[1]	U[1]
Tr4.11	P	M		P	P[1]		U	P[1]	P[6]	M?[1]		P[1]	U[1]
Tr5.20	P	P		P	P[1]		U	U[1]	P[6]	P[1]		P[1]	M?[1]
Tr5.21	P	P		P	P[1]		U	P[1]	P[6]	P[1]		P[1]	U[1]
EST1	P	P		P	P[2]		P						
EST2	P	P	P[1]	P	P[2]	P[5]	P	P[1]	P[1]		P[1]		
EST3	U		U[1]		P[2]	U[1]	U	U[1]	U[1]		U[1]		
EST4	?	P	P?[1]		P[2]	U[1]	P	P[1]	U[1]		M?[1]		
EST6	?				P[1]								
EST8	?	?	P[1]	?	P[1]	P[5]	U	M?[1]	M?[1]		M?[1]		
EST9	P	P	P[1]	?	?[2]	U[1]	P	P[1]	U[1]		P[1]		
EST12	P	M		P	?[2]	P[5]	P						
EST14					?								
EST15	U				P								
EST16					?								
Ecu1	P	?		P	P[3]		P		?				
Ecu2	P			P	P[3]		P		?				
Ecu3	P	P		U	U[3]		U						
Ecu4	P			U	P[3]		U		P?				
Ecu5	P			P	P[3]		P		P?				

References
[1]Gardner (1999); [2]Gardner et al. (1999, 2000); [3]Stow (2002); [4]Bull et al. (2001); [5]Bonnett (1999); [6]Cooper et al. (1997).

while those for *E. frerei* are contained in Cooper et al. (1997), Gardner et al. (1999, 2000), Stow (2002), and Fuller et al. (2005).

Information relating to the cross-amplification in *E. whitii* is contained in Chapple and Keogh (2005, in press). In this species PCR was performed in a 20 µl volume reaction, containing approximately 100 ng of template DNA, 2.5 pmol of the M13(-21) tailed sequence-specific forward primer, 10 pmol of the sequence-specific reverse primer, 10 pmol of a fluorescent dye-labelled M13(-21) universal primer (either 6-FAM, NED or PET; Applied Biosystems), 2 µl 10x PCR Buffer, 2 µl 10x Enhancer Solution (Gibco BRI Life Technologies), 3 mM $MgSO_4$, 2 mM dNTPs and 0.2 units of Platinum *Taq* DNA polymerase (Gibco BRI Life Technologies). A 'stepping down' PCR program was used to amplify each locus. Reactions were initially denatured at 94°C for 5 min, followed by an annealing step at 70°C for 15 sec and extension at 72°C for 1.5 min. This was followed by a further round of denaturation at 94°C for 30 sec, annealing at 70°C for 15 sec and extension at 72°C for 1.5 min. The annealing temperature was then dropped by 5°C in the next two rounds of cycling. This 'stepping down' in annealing temperature was repeated until a final annealing temperature of 35°C was reached. The next 50 cycles then were performed with this annealing temperature. A final extension step at 72°C was done for 7 min.

Details concerning the amplification of the loci in *E. saxatilis* are contained in Cooper et al. (1997), Gardner et al. (1999), and O'Connor and Shine (2003). PCR was performed in a 10 µl volume reaction, containing 5 µl of template DNA, 1x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPS, 400 nM of each primer, and 0.25 units of *Taq* DNA polymerase (all Sigma reagents). The PCR parameters for the Ecu primers followed Stow (2002). The PCR program for EST2, EST12 and Tr4.11 involved an initial denaturing step of 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a final extension step at 72°C for 10 min. The parameters for EST1 and Tr5.21 were similar except that the 35 cycles were substituted with one cycle with an annealing temperature of 55°C, followed by single cycles at 53°C, 51°C, and 49°C, with a further 30 cycles at 47°C. For Tr5.20, these cycles were replaced by 7 cycles at each of the following annealing temperatures: 55°C, 53°C, 51°C, and 49°C. For EST8 and EST9 the 35 cycles were replaced by annealing temperatures of 55°C (2 cycles), 53°C (2 cycles), 51°C (2 cycles), 49°C (2 cycles) and 47°C (20 cycles).

Within *Egernia* there are eight primer pairs that appear to perform well across all lineages (Table 1). The most successfully used loci are EST1, EST2, EST12, Tr5.20 and Tr5.21 with promising preliminary results from the recently developed Ecu1, Ecu2 and Ecu5 primers. The *Egernia* range of primers is highly polymorphic and extremely informative (Table 2). Tr4.11 however, ap-

TABLE 2. Cross-amplification of the available microsatellite primers in five *Egernia* species. Several loci were not trialled in each of the species: *E. cunninghami* (Tr3.8, EST14, EST16), *E. saxatilis* (Tr3.2, Tr3.8, Tr4.6, EST3-4, EST6, EST14-16), *E. whitii* (Tr3.8, Tr4.6, EST6, EST14-16), *E. frerei* (Tr3.2, Tr3.8, EST3, EST6, EST14-16, Ecu2, Ecu4-5) and *E. stokesii* (Ecu3). N = number of individuals trialled; NA = number of alleles; Ho = Observed heterozygosity; He = Expected heterozygosity; HWE P = *P*-value for Hardy-Weinberg equilibrium for each locus.

Locus	N	Size range	NA	Ho	He	HWE P	Notes
[1]E. cunninghami							
Tr3.2	189	161–269	20	0.961	0.928	NS	Linkage disequilibrium with EST12
Tr4.6	10	amplified				NS	Could not optimise [at](47, 55, 60)
Tr4.11	189	amplified	12	0.460	0.809	< 0.001	Sex-linked (females heterozygous)
Tr5.20	189	146–152	3	0.260	0.268	NS	High frequency of putative null alleles at one site (ca. 12%)
Tr5.21	189	79–145	18	0.887	0.875	NS	
EST1	189	209–337	20	0.940	0.883	NS	
EST13 (= EST2)	189	164–252	17	0.868	0.926	NS	
EST4	189	amplified	17	0.538	0.888	< 0.001	High frequency of null alleles (ca. 24%)
EST6	10	amplified				NS	Could not optimize [at](47, 55, 60)
EST8	10	amplified				NS	Could not optimize [at](47, 55, 60)
EST9	189	215–279	12	0.660	0.646	NS	
EST12	189	amplified	26	0.915	0.921	NS	Linkage disequilibrium with Tr3.2
Ecu1	161	144–194	17	0.883	0.902	NS	
Ecu2	161	154–196	13	0.848	0.843	NS	
Ecu3	161	220–272	11	0.784	0.825	NS	
Ecu4	161	76–288	29	0.825	0.924	NS	null alleles (ca. 9%)
Ecu5	161	120–164	17	0.894	0.904	NS	
E. saxatilis							
Tr4.11	28	134–137	2				Sex-linked
Tr5.20	277	127–191	29	0.693	0.894	<0.001	null alleles (ca. 13%)
Tr5.21	280	80–120	17	0.821	0.883	NS	
EST1	279	209–309	25	0.928	0.931	NS	
EST2	276	194–274	21	0.804	0.922	NS	
EST8	42	Amplified					Could not optimize
EST9	24	Amplified					Could not optimize
EST12	281	191–267	19	0.865	0.606	NS	
Ecu1	4	Amplified					
Ecu2	4	Amplified					
Ecu5	4	Amplified					
E. whitii							
EST1	127	226–314	20	0.787	0.928	NS	
EST2	127	188–280	20	0.969	0.923	NS	
EST4	127	123–179	14	0.858	0.874	NS	
EST9	15	259–277	4	0.400	0.579	NS	Putative null alleles
EST12	127	276–374	21	0.890	0.933	NS	
Ecu1	16	159–243	18?	0.813	0.915	NS	Di-repeat with 4 peak stutter
Ecu2	127	149–179	13	0.535	0.790	<0.001	null alleles (ca. 24%)
Ecu5	14	122–136	4	0.929	0.643	NS	
E. frerei							
Tr4.6	1	Amplified	1				
Tr4.11	28	130	1	N/A	N/A	N/A	Monomorphic
Tr5.20	229	118–126	6	0.489	0.485	NS	Di-repeat with stutter
Tr5.21	224	82–88	7	0.799	0.754	NS	Alleles differ by 1bp
EST1	225	188–266	30	0.804	0.935	NS	Alleles differ by 2bp
EST2	229	173–243	18	0.764	0.912	<0.001	Short allele dominance
EST4	225	108–120	4	0.280	0.261	NS	
EST8	85	104–176	?				Could not optimize
EST9	228	227–303	20	0.803	0.904	NS	
EST12	29	247	1				Monomorphic
Ecu1	16	141–151	3?				Could not optimize
Ecu3	15	229–281	9	0.800	0.883	NS	
E. stokesii							
Tr3.2	150	176–234	20	0.867	0.880	NS	Linkage disequilibrium with EST12
Tr5.21	50		4	0.292	0.2	0.0012	Null alleles (ca 13%), stutters
EST1	150	234–282	12	0.913	0.865	NS	
EST2	141	206–286	24	0.915	0.910	NS	

TABLE 2. Continued.

Locus	N	Size range	NA	Ho	He	HWE P	Notes
EST3	141	246–346	16	0.865	0.884	NS	Low frequency of null alleles, short allele dominance
EST4	150	141–189	11	0.793	0.850	NS	Low frequency of null alleles, short allele dominance
EST6	10	163–189	8	0.80			
EST8	149	101–141	8	0.799	0.816	NS	
EST9	10	235–263	9	0.60			High frequency of null alleles
EST12	149	288–336	13	0.846	0.879	NS	Linkage disequilibrium with Tr3.2
EST14	10	114–178	7	0			May contain null alleles
EST15	10	129–141	2	0.10			
EST16	10	156–184	5	0.40			May contain null alleles
Ecu1	50	111–186	4	0.612	0.22	<0.001	Null alleles (ca 28%), large alleles stutter.
Ecu2	50	132–170	8	0.683	0.62	NS	
Ecu4	50	64–72	3	0.578	0.58	NS	
Ecu5	50	107–121	11	0.87	0.8	NS	

[1] PCR conditions for optimized loci given in Stow et al. (2001) and Stow (2002)
[at] = annealing temperatures trialled (°C)

pears to be sex linked (females heterozygous) and Tr3.2 and EST12 appear to be directly linked (Table 2). Problems with short allele dominance and null alleles are present within the lineage but are not consistently related to a particular primer but rather the primer-species interaction (Table 2). Overall, the *Egernia* microsatellite loci are highly polymorphic and extremely informative for studies of sociality.

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LITERATURE CITED

BONNETT, M. P. 1999. The ecology, behaviour and genetic relationships of a population of *Egernia striolata*. Unpublished Honours Thesis, Flinders University, Adelaide, Australia.

BULL, C. M. 2000. Monogamy in lizards. Behav. Processes 51:7–20.

———, C. L. Griffin, M. Bonnett, M. G. Gardner, and S. J. B. Cooper. 2001. Discrimination between related and unrelated individuals in the Australian lizard *Egernia striolata*. Behav. Ecol. Sociobiol. 50:173–179.

CHAPPLE, D.G. 2003. Ecology, life-history, and behavior in the Australian scincid genus *Egernia*, with comments on the evolution of complex sociality in lizards. Herpetol. Monogr. 17:145–180.

———, AND J. S. KEOGH. 2005. Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. Mol. Ecol. 14:1215–1227.

———, AND ———. Group structure and stability in social aggregations of White's skink, *Egernia whitii*. Ethology 112:247–257.

COOPER, S. J. B., C. M. BULL, AND M. G. GARDNER. 1997. Characterization of microsatellite loci from the socially monogamous lizard *Tiliqua rugosa* using a PCR based isolation technique. Mol. Ecol. 6:793–795.

FULLER, S. J., C. M. BULL, K. MURRAY, AND R. J. SPENCER. 2005. Clustering of related individuals in a population of the Australian lizard, *Egernia frerei*. Mol. Ecol. 14:1207–1213.

GARDNER, M. G. 1999. A genetic investigation of sociality in the Australian group liviing lizard *Egernia stokesii*. Unpublished PhD thesis, Flinders University, Adelaide, Australia.

———, C. M. BULL, AND S. J. B. COOPER. 2002. High levels of genetic monogamy in the group-living Australian lizard, *Egernia stokesii*. Mol. Ecol. 11:1787–1794.

———, ———, ———, AND G. A. DUFFIELD. 2000. Microsatellite mutations in litters of the Australian lizard *Egernia stokesii*. J. Evol. Biol. 13:541–550.

———, ———, ———, AND ———. 2001. Genetic evidence for a family structure in stable social aggregations of the Australian lizard *Egernia stokesii*. Mol. Ecol. 10:175–183.

———, S. J. B. COOPER, C. M. BULL, AND W. N. GRANT. 1999. Isolation of microsatellite loci from a social lizard, *Egernia stokesii*, using a modified enrichment procedure. J. Hered. 90:301–304.

O'CONNOR, D., AND R. SHINE. 2003. Lizards in 'nuclear families': a novel reptilian social system in *Egernia saxatilis* (Scincidae). Mol. Ecol. 12:743–752.

STOW, A. J. 2002. Microsatellite loci from the Cunningham's Skink (*Egernia cunninghami*). Mol. Ecol. Notes 2:256–257.

———, AND P. SUNNUCKS. 2004a. High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. Mol. Ecol. 13:419–430.

———, AND ———. 2004b. Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. Mol. Ecol. 13:443–447.

———, ———, D. A. BRISCOE, AND M. G. GARDNER. 2001. The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. Mol. Ecol. 10:867–878.